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COMPARATIVE CYTOCHEMICAL AND STEREOLOGICAL STUDIES ON BLOOD PLATELETS FROM FIVE MAMMALIAN SPECIES $

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ABSTRACT

The present paper reports on comparative quantitative analyses of the thrombocytic content of adenine nucleotides (ATP, ADP; firefly luciferase technique), platelet-specific proteins (platelet factor 4, ß-thromboglobulin; radioimmunoassay), and a lysosomal enzyme (ß-glucuronidase; Fishman- and Szasz-technique), measured with the platelets from man, dog, pig, sheep, and cat. These data are faced with the results from morphometric and stereologic investigations (e.g. determination of volume- and numerical density) obtained from electronmicrographs of subcellular platelet structures, such as alpha-granules and dense bodies. Both organelles are known to be specific intrathrombocytic storage sites of the above mentioned substances.

Remarkable differences are described to exist between the platelets from the five species. An evident correlation, however, persisting between the thrombocytochemical and structural parameters is missing on the whole. Therefore it is pointed out that, at the moment, quantitative statements cannot be made upon the thrombocytic content of stored substances from the geometry of the platelet storage granules, alone

INTRODUCTION

Nowadays, pathophysiology is aware that platelets play an eminent role in the genesis and course of circulatory

dysfunctions. Therefore, great efforts are attempted to develop platelet-function controlling drugs. Preclinical tests of those substances usually are conducted with the "ordinary" laboratory animals. Extensive studies, however, have demonstrated considerable differences to exist among the platelets from different mammalian species. On the one hand, platelets were shown to react upon exogenous stimulation remarkably different, in a species-specific way (Tosch and Benner, 1979). On the other side, morphometric examinations revealed substantial discrepancies concerning the geometry of the subcellular composition of different mammalian platelets (Geeren and Benner, in press).

Since it is known that platelet function in-vitro can be activated by substances which in their turn normally are stored intrathrombocytically it is implied that the reactivity of these cells - expressed as their disposition towards metamorphosis, adhesion, release of cellular content, or aggregation - somehow is correlated with their contents of biogene amines (serotonin, adrenalin), adenine nucleotides and/or platelet specific proteins.

Purpose of the present paper was to biochemically quantitate some of the above mentioned substances in the platelets from five mammalian species and to compare these data with the results from electron-microscopic morphometry and stereology conducted on the storage organelles of the same cells.

MATERIALS AND METHODS

The cytochemical investigations were performed on samples of platelet-rich plasma (PRP) prepared from blood which was freshly withdrawn by venipuncture from the following species: man (n=8), dog (n=5), pig (n=4), sheep (n=3), and cat (n=2). The intracellular concentrations of the adenine nucleotides were measured using the bioluminiscence technique (Holmsen et al., 1966). This method is based upon the the dephosphorylation of ATP to AMP by a firefly luciferase system and the conversion of the splitted pyrophosphate to light-quanta taking place at the same time.

In more detail, PRP-samples (and - to serve as control - platelet-poor-plasma samples) were lysed by incubation at 98° C for the duration of 3 min. To 50 µl of the lysate 100

µl of a solution of luciferine-luciferase were sludge-injected; the resulting photoluminescence was registered using a photomultiplier (Baird-Atomic Filterfluorimeter FM 200 , photomultiplier 931 A).

The intracellular concentration of ß-glucuronidase was measured with two slightly differing techniques. The one, introduced by Fishman et al. (1967), utilizes the substrate complex phenolphthaleine-glucuronic-acid to quantitate the activity of ß-glucuronidase. This lysosomal enzyme acts on the substrate complex liberating the free phenolphthaleine. The intensity of the resulting color (red) is measured photometrically (Beckman, Spectrophotometer model 24) at a wave length of 550 nm and is proportional to the enzyme activity.

In this series of experiments the platelet-lysates from pig, sheep, and cat obviously showed no sign of enzymatic activity. Therefore a second method (Szasz, 1967) was employed in order to exclude that the unresponsiveness in these cases only was due to an inhibitory effect of the substrate phenolphthaleine on the activity of ß-glucuronidase as was assumed by Nimmo-Smith (1961). The Szasz-technique is based upon the same measuring principles as is the one described, but utilizes the substrate complex p-nitrophenole-ß-glucuronide.

Platelet factor 4 (PF4) and ß-thromboglobulin (ß-TG) were determined in PPP- and PRP-lysates (for these series the platelet membranes were disrupted by the addition of Triton X-100; 5 Vol %) employing a radioimmunoassay (RIA; Abbot, Wiesbaden, FRG). This technique depends upon the competitive displacement of the platelet-specific proteins by identical, but radioactive (125-J) labelled proteins from a limited number of receptors of a specific antibody. The amount of labelled protein linked with the antibody is converse proportional to the concentration of unlabelled protein in the sample.

The handling of the PRP samples for electron microscopic photography as well as the procedure for morphometric and stereologic evaluation of thrombocytic organelles is described in more detail in a second contribution (Geeren M, and Benner KU).

RESULTS AND DISCUSSION

Table 1 gives the results from the photoluminiscence- and stereologic determinations on platelets from the five mammals. The intracellular concentrations of ATP and ADP are compared with the values of volume density (Vv) and numerical density (Nv) of the thrombocytic DB. The Table demonstrates that there is no evident correlation between the intrathrombocytic levels of the adenine nucleotides and the geometry of their storage granules. The porcine platelets e.g. embodied the highest ATP- (23.9+/8.6) and ADP- (8.6+/-1.3, both in mg x 10^{-11} cells) concentrations measured. The volume fraction of DB in the pig platelets, however, was found to range in this confrontation at the bottom. On the other hand, Vv (3.5+/-3.2 um^0) and Nv (2.3+/2.2 um^{-3}) in sheep platelets ranged in the second place, whereas the thrombocytic nucleotide concentrations (ATP,ADP = 5.2+/-1.0, 6.0+/-4.7 mg x 10^{-11} cells) were the lowest in this group.

Table 1. Comparison of stereological and cytochemical data obtained on platelets from five mammalian species. The concentrations (in mg) of the adenine nucleotides listed refer to 10^{11} platelets; the stereological values - volume- (Vv) and numerical density (Nv) - were calculated for the dense bodies (DB; storage organelles of non-metabolic nucleotides). All values represent mean values and s.d.

SPECIES	n	ATP ($mg/10^{11}$ platelets)	ADP	Vv DB (um^0)	Nv DB (um^{-3})
man	8	13.3±5.1	7.2±3.1	2.9±3.2	3.3±3.4
dog	5	16.0 5.2	3.0 1.7	4.1 2.8	2.6 1.7
pig	4	23.9 8.6	8.6 1.3	1.7 1.4	1.8 2.0
sheep	3	5.2 1.0	6.0 4.7	3.5 3.2	2.3 2.2
cat	2	23.2 16.1	6.8 4.4	3.3 1.9	3.0 2.5

Nearly the same discrepancy becomes obvious when the intrathrombocytic content of ß-glucuronidase is opposed to

the Vv- and Nv-values of the alpha-granules which are assumed to store (among other substances) the lysosomal enzymes (Fig. 1). Again, sheep platelets were observed to develop the largest volume fraction of these subcellular structures measured; their ß-glucuronidase activity, however, was comparatively low (immeasurable with the Fishman-technique). In contrast, human platelets displayed a relative high enzymatic activity at a moderate volume fraction of alpha-granules.

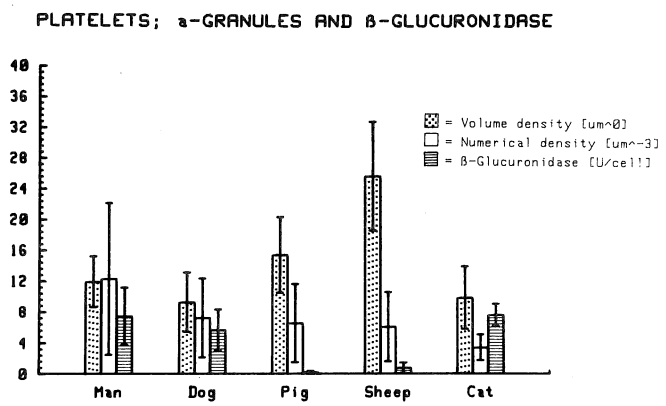

Fig 1. Comparison of stereological and cytochemical examinations obtained on the platelets from five mammalian species. The activity of ß-glucuronidase (in U/cell) was determined with the Szasz-technique. Mean values and s.d.

The disagreement between the geometry of the storage organelles and the concentrations of substances localized in those organelles may rest upon several reasons. It could be assumed e.g. that just the proportional fraction of the non-metabolic platelet adenine nucleotides differs substantially in the species investigated. Only this portion is reported to be stored in the DB (White and Gerrard, 1980) and represents in human platelets approx. 70% of the total intracellular adenine nucleotide pool. On the other side, the package densities of the substances stored within the platelet granules may vary from one species to the other.

The platelet specific proteins - also said to be localized in organelles quite similar to alpha-granules - not at all can be recruited for a comparison, as may be

taken from Table 2. With the RIA assay used ßTG was provable exclusively in the platelets from man, and PF4 only in human and feline thrombocytes. This observation can be discussed to depend upon one of the following reasons: firstly, the specific protein actually is missing in all platelets other than those from man (and cat), or secondly - and this is more plausible - human platelet-specific antigen/antibody complexes differ in their amino-acid sequences from those of the other species. The consequence would be that the radioactive labelled human proteins used for the RIA cannot be attached to the receptors of the thrombocytic antibodies of the other mammals.

Table 2. Results of comparative determinations of the platelet specific proteins platelet factor 4 (PF4) and ß-thromboglobulin (ßTG; both in µg x 10^{-9} cells) using a radioimmunoassay.

SPECIES	n	PF4 (µg/10^9 cells)	ßTG (µg/10^9 cells)
man	8	52.00 +/- 14.00	121.8 +/- 35.8
dog	5	0.82 +/- 0.26	-
pig	4	0.70 +/- 0.13	-
sheep	3	0.80 +/- 0.40	-
cat	2	52.50 +/- 20.50	-

In conclusion the study in hand demonstrates that morphometry and stereology of thrombocytic subcellular structures at the moment are unable to furnish with realistic quantitative informations about specific substances stored in those granules and vesicles.

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